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16. ABSTRACT

A committee of scientists was established by the U.S. Environmental Protection Agency to appraise the current state of aneuploidy test methodology, to compile and analyze published data on the chemical induction of aneuploidy, and to provide guidance for additional test development and validation. The committee was organized into 9 working groups, representing different eukaryotic organisms ranging from fungi to whole mammals. The Environmental Mutagen, Carcinogen, and Teratogen Information Program at Oak Ridge National Laboratory identified and provided literature to the working groups. The 9 reports were prepared by the individual working groups. The reports include: (1) descriptions of the available testing approaches and general principles to be followed for protocol development, (2) criteria for evaluating data, and (3) recommendations for future test development and basic research. The evaluated results are presented in tables. For the positive and negative calls, the lowest effective dose tested or the highest ineffective dose tested, respectively, and the range of concentrations tested are provided. An overall summary report was prepared that summarizes the conclusions and recommendations of the 9 working groups and the chemical data base. It is hoped that these reports will provide a framework for further test development and validation of aneuploidy assays.

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The detection of chemically induced aneuploidy in *Saccharomyces cerevisiae*: an assessment of mitotic and meiotic systems *

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Summary

Several systems have been evaluated for their ability to detect aneuploidy. Chromosome gain can be detected in mitotic haploid cells as well as meiotically derived haploid spores. Both chromosome gain and loss are detectable in mitotic diploid cells. Several chemicals have been identified that clearly induce aneuploidy in at least one or more of the systems.

(1) Introduction

Great interest has been generated in recent years in the identification of chemicals that affect the proper segregation of chromosomes at mitosis and meiosis; of particular concern are those agents that induce aneuploidy but do not affect other end points such as recombination or intragenic mutation. The potential genetic hazard of such chemicals may go undetected in the commonly used battery of available test systems.

The yeast *Saccharomyces cerevisiae* has been widely employed for investigations of basic genetic mechanisms, including chromosome malsegregation, because of its extensive genetic characterization and its relative ease of manipulation in the laboratory. For these reasons, *S. cerevisiae* has been used as a practical and definitive tool to analyze the ability of chemicals to induce gene mutations, recombination — both gene conversion and crossing-over — as well as aneuploidy.

Yeast is particularly well suited for studies of chromosome malsegregation because aneuploidy potentially can be evaluated in terms of chromosome gain and loss in mitotically growing or meiotically developing cells. The chromosome number of normal haploid and diploid strains of *S. cerevisiae* is quite stable. Aneuploid strains have been constructed (Bruenn and Mortimer, 1970; E.M. Parry and Cox, 1970; Shaffer et al., 1971) and apparently are sufficiently stable to permit their use in a number of basic genetic studies. Advantage has been taken of trisomics to show linkage between two distant groups of genes on one chromosome (Kawasaki, 1979) and to assign newly identified genes to a particular chromosome (Hilger and Mortimer, 1980; Mortimer and Hawthorne, 1973; Wickner, 1979). Triploid strains have been used to study the frequency of disomics recovered after meiosis (Campbell et al., 1981; E.M. Parry and Cox, 1970). Aneuploid strains have also proven useful in the isolation of mutants defective in mitotic (Rodarte-Ramon and Mortimer, 1972) or meiotic (Roth and Fogel, 1971) recombination.

The stability of chromosome number in the genome clearly is under genetic control. The presence of mutant alleles of any of a number of genes leads to high frequencies of numerical chromosomal abnormalities. In one case, a mutant controlling bisexual behavior led to nonrandom chromosome loss (Haber, 1974; Liras et al., 1978). A similar situation has been reported for certain fatty-acid-synthesis mutants (Henry and Fogel, 1971; Culbertson and Henry, 1973). The recently isolated *dis* mutants control mitotic and meiotic chromosome segregation (Rockmill and Fogel, in preparation). High rates of random chromosome loss occur in diploid strains homozygous for *rad52*, a radiation-sensitive mutant (Mortimer et al., 1981). Aneuploidy is also one characteristic of strains containing suppressors of *cdc14* (Kawasaki, 1979).

Other factors and treatments are known that lead to numerical chromosomal abnormalities. Although it is not within the scope of this article to present details, mention must be made that ionizing radiation and UV light induce aneuploidy (J.M. Parry et al., 1979b; J.M. Parry and Sharp, 1981; Mortimer et al., 1981; Esposito et al., 1982) and that thymidylate stress leads to chromosome loss (Kunz and Haynes, 1982), as well as to increased recombination. Evidence has been presented that suggests a relationship between increased recombination and aneuploidy, especially when recombination occurs in a region near the centromere (Campbell and Fogel, 1977; Campbell, 1980; Liras et al., 1978; Campbell et al., 1975).

Additional support is provided by observations that the frequency of aneuploidy is enhanced in diploids homozygous for mutations that result in faulty recombination events (Kunz and Haynes, 1982). Thus, it seems likely that treatments that enhance recombination may also increase the frequency of aneuploidy.

Among the early work on chemical induction of aneuploidy in *S. cerevisiae* are observations that *para*-fluorophenylalanine induces chromosome malsegregation (Emeis, 1966; Stromnaes, 1968). Based on these and subsequent observations, it has become clear that chemicals that induce aneuploidy might constitute a human genetic hazard. This perspective has prompted a number of investigators to develop systems for the detection of induced aneuploidy, based on relatively simple and rapid microbial methodologies that could be applied to screening large numbers of chemicals. Since recent efforts have resulted in the beginnings of a data base with a limited number of tested chemicals, it is now appropriate to assess our current understanding of the process of aneuploidy in yeast, to present an appraisal of available detection schemes and their use for testing chemicals, and to suggest some future directions for research in this area. We have examined 35 papers which are related to aneuploidy detection in yeast. Based on criteria presented below, data in 19 papers were analyzed for evidence of chemically induced aneuploidy.

(2) Description of available test systems

(2.1) Mitotic chromosome loss

In general, aneuploidy detection systems that are based on chromosome loss in *S. cerevisiae* detect the expression of multiple recessive markers resulting from the loss of a homologous chromosome carrying the corresponding dominant alleles. It is imperative that these markers be located on both sides of the centromere; otherwise a single recombinational event could produce the phenotype expected for chromosome loss. Two such recessive markers may be suffficient if it can be demonstrated that the frequency of double recombination and/or mutation events is sufficiently low so as not to interfere with the identification of the relatively rare chromosome loss events. It must also be demonstrated that other possible events do not mimic the recessive phenotypes. It is obvious that three or more such markers will give much greater assurance in the result than afforded by two.

(2.1.1) Strain D6

One of the first strains for the routine detection of chromosome loss in yeast was developed by J.M. Parry and Zimmerman (1976). Strain D6 has since been used extensively to detect aneuploidy induction by chemicals (Ferguson and Parry, 1984; Kelly and Parry, 1983; J.M. Parry, 1977, 1982; J.M. Parry and Sharp, 1981; J.M. Parry and Zimmerman, 1976; J.M. Parry et al., 1979a, b, 1981). It has the following genotype:

Chromosome III $\frac{his4}{+} \theta \frac{MATa}{MAT\alpha}$

Chromosome VII $\frac{ade3}{+} \theta \frac{leu1}{+} \frac{trp5}{+} \frac{cyh^R2}{+} \frac{met13}{+}$

Chromosome XV $\frac{ade2\text{-}40}{ade2\text{-}40}$

This strain forms red adenine-requiring colonies because of the homoallelic mutation at the *ADE2* locus. It will not grow on complete medium containing 2 ppm cycloheximide because of the presence of the wild-type *CYH2* allele. Loss of one copy of chromosome VII, leaving the homologue containing the multiple recessive recessive alleles, will result in the *ade3 cyh2* genotype. Such cells will grow on cycloheximide medium, but the colonies will be white and adenine- (and histidine-) requiring because of expression of *ade3*. These white cycloheximide-resistant colonies will also express the centromere-linked gene *leu1* (leucine requirement) and the more distal *trp5* (tryptophan requirement) and *met13* (methionine requirement) if the entire chromosome has been lost. Other genetic events, such as mutation, mitotic recombination, or partial chromosome loss, usually will be detected as red cycloheximide-resistant colonies (see below, Section 2.1.3).

(2.1.2) Strain D61.M

More recently, a strain similar to D6 has been

described by Zimmerman and Scheel (1984) and designated D61.M. There are, at present, 3 published reports of its use to test chemicals for aneuploidy (Zimmermann and Scheel, 1984, Zimmermann et al., 1984, 1985). The relevant genotype for detection of chromosome VII loss is:

Chromosome VII $\frac{ade6}{+} \frac{leu1}{+} \frac{+}{trp5} \frac{cyh^R2}{+}$

Chromosome XV $\frac{ade2\text{-}40}{ade2\text{-}40}$

Strain D61.M also forms red colonies and is sensitive to cycloheximide. Loss of the copy of chromosome VII containing the wild-type alleles *ADE6*, *LEU1*, and *CYH2* will result in expression of the recessive *ade6* (colonies are white and adenine-requiring) and *cyh*^{R}*2* mutations (resistance to cycloheximide). The *ade6* is much closer to the centromere than is the *ade3*. As with D6, presumptive monosomics (white cycloheximide resistant) can be confirmed by testing for *leu1* on leucine-deficient medium. Strain D61.M is also homoallelic for *ilv1-92* (isoleucine requirement), useful for testing reverse mutation induction. As discussed below, it is essential to test for the *leu1* mutation, since cyh^R white colonies could have arisen by recombination events and mutations at the *ADE3* or *ADE6* locus, reverse mutations, or even respiratory-deficient mutations (petites).

(2.1.3) Potential problems with strains D6 and D61.M

Following the construction of strain D6, J.M. Parry and Zimmerman (1976) were able to demonstrate on genetic grounds that the majority of white cycloheximide-resistant colonies arising spontaneously were due to monosomy. As judged by tests for linked markers, approximately 60% of the white cycloheximide-resistant colonies apparently were due to chromosome malsegregation. Similar results were obtained with UV light and ionizing radiation. The remainder must be considered to have arisen by other genetic mechanisms. The possibility of cycloheximide-resistant white colonies arising by other genetic events must therefore be determined. Typically, the control frequency of red cyh^R colonies is 10^{-4}–10^{-5} with white cyh^R colonies about 200-fold less; the total number of colonies per plate of cycloheximide-containing medium is approximately 200–600. Increases in cyh^R colonies could occur by a forward mutation of *CYH2* either to a nonfunctional state or to resistance by mutations at a limited number of sites (Brusick, 1972; Cooper et al., 1967; Rao and Grollman, 1967; Siegel and Sisler, 1965; Sisler and Siegel, 1967) or by recombination. Among colonies which are cyh^R, whites can arise in various ways. They could occur by reversion of *ade2-40* in the cyh^R cells; we consider this unlikely since the reverse mutation is likely to be both site-specific and mutagen-specific and therefore somewhat rare. However, forward mutation at the wild-type *ADE2* locus (or *ADE6* in strain D61.M) can yield white colonies, and this event is likely to occur more frequently than reversion because multiple sites for mutation are available; it may be sufficiently frequent to be detected in the test. High spontaneous and induced rates for forward mutation have been reported (Eckardt and Haynes, 1977). In addition, Roman (1956) has reported strong selection for cells carrying both an *ade2* mutation and an *adeX* mutation (i.e., *ade3, ade6,* etc.) that results in adenine-requiring white colonies. Since there is a requirement for growth during treatment, selection may be important. A small increase in the frequency of white cycloheximide-resistant colonies, caused by selection and/or induction, might be enough to yield an apparent positive response for aneuploidy. The appearance of cyh^R white colonies could also be due to multiple recombinational events. The simplest way to ensure against these possible problems is to test for *leu1* or other auxotrophic markers on chromosome VII.

Presumptive aneuploids (i.e., white, cyh^R) can also arise by the induction of petite mutations in pre-existing cyh^R cells, because petite mutants may not develop the red color of *ade2* mutants (Eckardt and Haynes, 1977). Petite mutants occur spontaneously in *S. cerevisiae*, and as much as 1% of the population can be petite. Among a population of 200–600 cycloheximide-resistant colonies per plate (typical for experiments with D6 or D61.M), a few petites would be expected, approximating the spontaneous frequency of chromosome VII monosomics in the same population. If petites were induced by chemical or physical treatment, the

number of petites could fluctuate 2–3-fold. In recent experiments with D61.M, Mayer and Zimmerman (unpublished) observed that cytoplasmic petite mutants mimicked the white cycloheximide-resistant colony phenotype of presumptive aneuploids. Because such colonies would be confused with those arising from malsegregation, testing for the additional *leu1* marker is essential to confirm monosomy.

(2.1.4) Strain LBL 1

A third strain, designated LBL 1, also detects chromosome VII loss (Esposito et al., 1982) and has been used to determine the effects of UV. Strain LBL 1 is a haploid that is disomic for chromosome VII and has the following genotype:

Chromosome III *MATa*

Chromosome VII $\frac{cly8\ ade6}{+\ +} \underset{leu1}{0} \frac{+\ trp5\ cyh2\ ade5}{+\ +\ +\ +}$

Chromosome XV *ade2*

Cultures are grown and maintained on a medium lacking leucine and tryptophan, which ensures the disomic condition of chromosome VII (haploid segregants that lose either copy of chromosome VII cannot grow without the required supplement). The strain requires adenine and accumulates red pigment due to the *ade2-1* mutation on chromosome XV. It is sensitive to cycloheximide due to the wild-type allele *CYH2* on one copy of chromosome VII. The normal phenotype of strain LBL 1 is adenine-requiring red cycloheximide-sensitive colonies that are leucine- and tryptophan-independent.

Loss of the copy of chromosome VII that carries the wild-type alleles *CLY8, ADE6, TRP5, CYH2,* and *ADE5* results in colonies that require adenine but are white and cycloheximide-resistant. In addition, such clones require tryptophan and will not grow at 36°C because of *cly8*. Because either the *ADE5* or *ADE6* allele would result in the white colony color, chromosome malsegregation resulting in the loss of both alleles must be confirmed by complementation tests using *ade5* and *ade6* tester strains. The multiple markers carried on chromosome VII permit detection of coincident recombinational events in several intervals.

(2.1.5) Strains g632 and g551

Kunz and Haynes (1982) have described two similar diploid strains constructed by Dr. J.C. Game that detect the occurrence of mitotic chromosome loss. The relevant genotypes are:

g632: $\frac{CAN1\ ura3}{can1\ ura3} \underset{hom3\text{-}10}{0} \frac{HOM3\ his1\text{-}7\ rad54\text{-}3}{his1\text{-}1\ rad54\text{-}3}$

g551: $\frac{CAN1\ URA3}{can1\ ura3} \underset{his1\text{-}1}{0} \frac{his1\text{-}7\ TRP2\ rad57\text{-}1}{trp2\ rad57\text{-}1}$

Flanking the centromere of chromosome V in strain g632 are recessive alleles for canavanine resistance (*can1*) and for homoserine dependence (*hom3*), which would both be expressed in the event of loss of the homologous chromosome carrying the wild-type alleles. In addition, two heteroalleles at the *HIS1* locus permit observation of gene conversion, which is not possible if only one copy of chromosome V is present. Strain g551 carries a similar configuration of recessive markers for canavanine resistance (*can1*), uracil requirement (*ura3*), and tryptophan requirement (*trp2*). These strains also carry the radiation-sensitive alleles *rad54-3* or *rad57-1*, known to cause higher frequencies of spontaneous and X-ray-induced chromosome loss (R.K. Mortimer and D. Schild, personal communication, cited in Kunz and Haynes, 1982). It is unknown whether their presence would alter test data. These particular alleles are, however, temperature-conditional and so it is possible to use these strains at the permissive temperatures.

(2.1.6) Strains XD72 and XD79

A system recently described by Dixon (1983) for the detection of mitotic chromosome loss differs from that of Parry and Zimmerman in that aneuploidy is detected by the coincident loss of selectable markers on both sides of a centromere rather than just one side. Centromere-linked dominant suppressors (*SUP7* and *SUP4*) are present on both sides of the chromosome X centromere:

$$\frac{MATa}{MAT\alpha}\ \frac{ade2\text{-}1}{ade2\text{-}1}\ \frac{his5\text{-}2}{his5\text{-}2} \underset{lys1\text{-}1}{0} \frac{lys1\text{-}1}{lys1\text{-}1}\ \frac{can1\text{-}100}{can1\text{-}100}$$

$$\frac{+\ \ SUP7}{ura2\text{-}1\ +} \underset{ilv3\text{-}1}{0} \frac{+\ \ SUP4\ trp1\text{-}1\ +}{+\ +\ asu}$$

Cells lacking the suppressor-containing chromosome can be selected in two ways: on medium that does not support growth of strains with multiple suppressors or by resistance to canavanine (*SUP4* and *SUP7* suppress the *can1-100* mutation, rendering the cells sensitive to canavanine). The strains were developed to allow the confirmation of aneuploidy using a centromere-linked marker. On subsequent testing for the centromere-linked *ilv3* marker, many of the putative aneuploid cells appeared to have arisen through recombinational processes (Dixon, 1983).

The presence of the dominant suppressors *SUP4* and *SUP7* results in the suppression of *can1-100* and therefore renders the cells canavanine-sensitive. The *ade2-1, his5-2,* and *lys1-1* are also ochre mutations so that the presence of the suppressors renders the cells capable of growing on medium lacking histidine, adenine, and lysine. These mutations enable the monitoring of other suppressor artifacts. The *ura2-1* and *ilv3-1* mutations are also suppressible by *SUP4* and *SUP7*; loss of the suppressor-containing chromosome results in the ability of cells to grow on ethylene glycol and also on canavanine.

(2.2) Mitotic chromosome gain

(2.2.1) Strain D6

Strain D6, described in Section 2.1.1, has also been used to detect disomy for chromosome VII (J.M. Parry et al., 1979b). If nondisjunction of chromosome VII occurs during mitosis, the result will be one daughter cell that is monosomic and the other that is trisomic. If the two daughters have not separated before plating, the combination appears as a white and red sectored colony on nonselective medium. The white portion should express all of the recessive alleles. After sporulation, the red portion should generate disomic segregation patterns in the spore tetrads for all of the dominant alleles. The procedure is complicated by the frequent occurrence of incomplete asci and poor spore viability (J.M. Parry et al., 1979b), probably caused by multiple chromosome losses and gains (Kawasaki, 1979; Wood, 1982; Mortimer et al., 1981). The procedure is, moreover, too complex and tedious to be useful for screening chemicals, but development of a more practical system could be based on the above observations.

(2.2.2) Strain BR1669

Until recently, it was not possible to study various kinds of aneuploidy in a unified system. As discussed above, mitotic events were detected by the loss of a chromosome, thereby uncovering recessive markers. Meiotic aneuploidy was detected by the appearance of disomy among the haploid products, the chromosomes having several recessive markers in repulsion so that only disomic cells that were prototrophic for all the markers would grow. The latter system could detect only a fraction of malsegregation events.

An alternative system has been developed that utilizes gene dosage in relation to ploidy to select for cells that are aneuploid (Whittaker et al., 1984).

$$\frac{his4\text{-}290}{his4\text{-}280}\ \frac{MATa}{MAT\alpha}\ \frac{cyh10}{+}$$

$$\frac{arg4\text{-}8}{arg4\text{-}8}\ \frac{thr1\text{-}4}{+}\ \frac{cup1\text{-}1}{cup1\text{-}1}\ \frac{met13}{+}\ \frac{ade2\text{-}1}{ade2\text{-}1}$$

Fogel and his colleagues have shown that cells with the mutation *arg4-8* are temperature-sensitive for growth on medium lacking arginine. Cells are auxotrophic for arginine at temperatures above 30°C but are able to grow at 25°C. Cells that have an extra copy of chromosome VIII (3 copies of *arg4-8* per cell) are able to grow at 30°C but not at 35°C. The reason for this growth at the restrictive temperature may be attributed to the presence of additional, albeit faulty, *ARG4* protein (arginosuccinate lyase) relative to total cellular protein. The result would be leakiness at higher temperatures. Similarly, the *cup1* gene, when present in an extra chromosome, results in greater resistance to copper in the medium than cells that have a euploid number of chromosomes. Since *arg4-8* and *cup1* are linked, malsegregation detected with one marker can be confirmed with the aid of the other marker.

Using this system, it has been possible to measure chromosome gain in both haploid and diploid mitotically growing cells and in meiotically developing cells. Another unique feature of this system

is that it enables the detection of cells that are actually in an aneuploid condition. As shown by Zimmermann et al. (1985) with the D61.M mitotic chromosome loss system, the cells that are recovered after selection for a chromosome loss event are usually euploid, suggesting that there is a strong selection for euploidy among cells that are deficient in a chromosome.

(2.3) Meiotic chromosome gain

The strain BR1669 described above can also be used for efficiently detecting nondisjunction during meiosis (Whittaker et al., 1984). Other strains, D9J2 and DIS13, detect among the meiotic products those spores that have homologous chromosomes with complementary markers.

(2.3.1) Strain D9J2

A diploid with a multiply marked chromosome VII was developed in order to detect disomic spores produced during the first meiotic division (J.M. Parry, 1977; J.M. Parry et al., 1979a,b). Strain D9J2 has the following genotype:

Chromosome V $\frac{can1}{+}$ Chromosome XI $\frac{ura1}{+}$

Chromosome VII

$$\frac{ade3 \; + \; \overset{0}{} \; leu1 \; + \; cyh2 \; + \; aro2 \; + \; ade5}{+ \; ade6 \; + \; trp5 \; + \; met13 \; + \; lys5 \; +}$$

Normal haploid spores resulting from meiosis cannot grow in minimal medium because of the multiple auxotrophic requirements carried by alleles on each copy of chromosome VII. Spores resulting from nondisjunction of chromosome VII at the first meiotic division can grow on minimal medium containing uracil. Half of such spores are expected to be resistant to canavanine. Diploid cells that fail to sporulate are sensitive to canavanine.

Cells are treated either prior to or during sporulation in acetate medium. After treatment and sporulation, spores from disrupted asci are plated onto minimal medium containing uracil and canavanine to detect disomic spores. Parry and co-workers have presented evidence that *para*-fluorophenylalanine, UV light, and X-rays produce a dose-related increase in the frequency of disomic spores, using the method outlined here (J.M. Parry, 1977; J.M. Parry et al., 1979a,b). Further details of treatment conditions can be found in J.M. Parry et al. (1979a,b). As discussed in the following section, some spores may be diploid rather than haploid.

(2.3.2) Strain DIS13

This strain was constructed by Sora et al. (1982) for the detection of meiotic aneuploidy. The basis for detection is similar to that described above for D9J2, only in this case, there are heterozygous genes on chromosome V.

III $\frac{MATa}{MAT\alpha}$

V $\frac{can1}{CAN1} \; \frac{URA3}{ura3} \; 0 \; \frac{HOM3}{hom3} \; \frac{his1}{HIS1} \; \ldots$

$\ldots \; \frac{ARG6}{arg6} \; \frac{ilv1}{ILV1} \; \frac{TRP2}{trp2} \; \frac{met5}{MET5}$

VII $\frac{cyh2}{CYH2} \; \frac{leu1}{LEU1}$

XV $\frac{ade2}{ADE2}$

Pure spore suspensions are prepared and cells are plated onto medium lacking all the supplements corresponding to the markers on chromosome V. This selects for 3 categories of cells: (1) spores that are disomic for chromosome V; (2) haploid spores, which because of several recombinational events are prototrophic for all the markers (this is very unlikely); or (3) diploid spores. Because of the opportunity for recombination during meiosis, many aneuploid or diploid spores are not detected. As discussed by Sora et al. (1982), the detection of spores that are disomic for chromosome V requires extensive genetic analysis. The total spore colonies growing on the multiple drop-out medium are determined; diploid spores are estimated and subtracted from the total to yield the estimated number of disomic spores. The frequencies of disomic and diploid spores detected are similar. Verification of disomy requires subsequent crosses and dissection. This system has been very useful for detecting diploid spores after treatment with such agents as caffeine, cyclophosphamide and

bleomycin (Sora et al., 1982, 1983), as well as disomic spores (Table 1).

(3) Evaluation of chemical tests

(3.1) Criteria

We have established minimal criteria that must be met to adequately evaluate the results of published studies. Some of these criteria seem obvious and generally are applicable to any system and investigation. Others are not so obvious, may apply uniquely to a particular test system, and were often derived as a result of experience with the test system itself. Only the data in published papers have been evaluated.

(3.1.1) Treatment other than chemicals

Because our review is directed toward the effects of individual chemicals we excluded treatments such as UV light, ionizing radiation, and heat (J.M. Parry et al., 1979b; Evans and Parry, 1975) unless the report had direct bearing on the scientific basis of the test system itself.

(3.1.2) Basic genetic studies

Although we perceive such reports as relevant and their contents of great interest to our review, the methodology employed in a number of studies was often too involved and cumbersome to be readily applicable in routine screening situations. While we might not recommend the procedures for routine screening, we did accept the results of those investigations involving extensive and complicated analyses.

(3.1.3) Positive and negative controls

It is obvious adequate controls are the corner-

TABLE 1

CONCLUSIVE ANEUPLOIDY TEST RESULTS [a] IN *Saccharomyces cerevisiae*

Compound	Strain	CAS Registry No.	Mitotic chromosome loss	Meiotic chromosome gain	Response	Lowest effective dose tested	Reference
Acetone	D61.M	67-64-1	X		+	4.8%	Zimmermann et al., 1984
5-Azacytidine	D61.M	320-67-2	X		−	5 mg/ml [b]	Zimmermann and Scheel, 1984
Benomyl	DIS13	17804-35-2		X	+	20 μg/ml	Sora et al., 1982
Cyclophosphamide	DIS13	50-18-0		X	+	2 mg/ml	Sora et al., 1983
Ethyl methane-sulfonate	D6	62-50-0	X		+	2%	J.M. Parry et al., 1979a
p-Fluorophenyl-analine	Several	60-17-3	X		+	–	Stromnaes, 1968
Methyl-benzimid-azole-2-carbamate (MBC or bavistan)	Several	10605-21-7	X		±	20 μg/ml	Wood, 1982
	D61.M		X		+	10 μg/ml	Zimmermann et al., 1984
Methyl methane-sulfonate	DIS13	66-27-3		X	±	3 μg/ml	Sora et al., 1982
Mezerein	D6	34807-41-5	X		−	10 μg/ml [a]	J.M. Parry et al., 1981
Oncodazole (nocodazole)	D61.M	31430-18-9	X		+	2.5 μg/ml	Zimmermann et al., 1984
12-*O*-Tetradecanyl-phorbol-13-acetate (TPA)	D6	16561-29-8	X		+	0.1 μg/ml	J.M. Parry et al., 1981

[a] +, positive induction of aneuploidy; −, no induction; ±, weak induction.
[b] Highest ineffective dose.

TABLE 2

INCONCLUSIVE ANEUPLOIDY TEST RESULTS [a]

Chemical	CAS Registry No.	Dose range (μg/ml unless specified)	Comments	Reference
2-Acetylaminofluorene	53-96-3		c,d	5
4-Acetylaminofluorene	28322-02-3		c,d	5
Adriamycin [a]	23214-92-8	0.25–2.5	b	10
3-Aminotriazole	61-82-5		c,d	5
Amphotericin B [a]	1397-89-3	0.05–1	b	9
Anthracene	120-12-7		c,d	5
Anthralin	1143-38-0		c,d	7
Ascorbic acid	50-81-7		c,d	5
Atenolol [a]	29122-68-7	1–8 μM	b,c,d	8
Auramine	2465-27-2		c,d	5
Azoxybenzene	495-48-7		c,d	5
Benzidine	92-87-5		c,d	5
Benzo[*a*]pyrene	50-32-8		c,d	5
Benzo[*a*]pyrene	50-32-8	50–500	c,d	3
Benzyl chloride	100-44-7	25–200	b,c,d	4
Bleomycin [a]	11056-06-7	1–100	b	10
Butter yellow	60-11-7		c,d	5
γ-Butyrolactone	96-48-0		c,d	5
Caffeine [a]	58-08-2	1.25–12.5 mM	b,c,d	8
Caffeine	58-08-2	125–1000	c,d	6
Chenodeoxycholic acid	474-25-9	12.5–1000	d	2
Chloroform	67-66-3		c,d	5
4-Chloromethyl-biphenyl	1667-11-4	25–200	b,c,d	4
Cholic acid	81-25-4	50–1000	d	2
Cyclophosphamide	50-18-0	50–500	c,d	3
Cyclophosphamide	50-18-0		c,d[e]	5
Deoxycholic acid	83-44-3	50–1000	d	2
Diethylstilbestrol	56-53-1		c,d	7
Diethylstilbestrol	56-53-1		c,d	5
15,16-Dihydro-11-methylcyclopenta[*a*]phenanthren-17-one	5836-85-1	50–500	c,d	3
9,10-Dimethylanthracene	781-43-1		c,d	5
Dimethylcarbamoyl chloride	79-44-7		c,d	5
Dimethylformamide	68-12-2		c,d	5
DMSO	67-68-5	2.5%	d	2
DMSO	67-68-5	5.0%	d	2
Dinitrosopentamethylene tetramine	101-25-7		c,d	5
Diphenylnitrosamine	86-30-6		c,d	5
Epichlorhydrin	106-89-8		c,d	5
DL-Ethionine	67-21-0		c,d	5
Ethylenethiourea	96-45-7		c,d	5
p-Fluorophenylalanine	60-17-3	0.3%	c,d	1
p-Fluorophenylalanine	60-17-3	50–500	a,c,d,	6
Glycodeoxycholic acid	360-65-6	25–3000	d	2
Hexamethylphosphoramide	680-31-9		c,d	5
Hydrazine sulfate	10034-93-2		c,d	5
4-Hydroxymethyl-biphenyl	3597-91-9	25–200	b,c,d	4

TABLE 2 (continued)

INCONCLUSIVE ANEUPLOIDY TEST RESULTS *

Chemical	CAS Registry No.	Dose range (μg/ml unless specified)	Comments	Reference
Iodoacetic acid	64-69-7		c,d	7
Isopropyl *N*-(3-chloro-phenyl) carbamate	101-21-3		c,d	5
Lauric acid	143-07-7		c,d	7
Lithocholic acid	434-13-9	50–1000	d	2
Mercurochrome	129-16-8	25–350	c,d	6
Methionine	63-68-3		c,d	5
3-Methyl-4-nitroquinoline-*N*-oxide	14073-00-8		c,d	5
4-*O*-Methyl-TPA	57716-89-9		c,d	7
Methyl orange	547-58-0		c,d	5
Mitomycin C	50-07-7	5–40	c,d	6
Mitomycin C [a]	50-07-7	25–30	b	10
MOCA	101-14-4		c,d	5
1-Naphthylamine	134-32-7		c,d	5
2-Naphthylamine	91-59-8		c,d	5
2-Naphthylamine	91-59-8	50–500	c,d	3
4-Nitroquinoline *N*-oxide	56-57-5		c,d	5
N-Nitrosomorpholine	59-89-2		c,d	5
Oleic acid	112-80-1		c,d	7
4 α-PDD	27536-56-7		c,d	7
Phorbol	17673-25-5		c,d	7
Phorbol-13-acetate	32752-29-7		c,d	7
Phorbol-12,13-didecanoate	24928-17-4		c,d	7
Propanolol [a]	525-66-6	7.5–25 μM	b,c,d	8
β-Propiolactone	57-57-8		c,d	5
Pyrene	129-00-0		c,d	5
Saccharin	81-07-2		c,d	7
Saccharin	81-07-2	200–1600	c,d	7
Safrole	94-59-7		c,d	5
Sodium chloride [a]	7647-14-5	3–20 mg/ml	b	10
Stearic acid	57-11-4		c,d	7
Stilbestrol dipropionate	130-80-3	50–500	c,d	6
Sulfacetamide	144-80-9	125–5000	c,d	6
Taurodeoxycholic acid	516-50-7	50–3000	d	2
12-*o*-Tetradecanoylphorbol	20839-06-9		c,d	7
3,3′,5,5′-tetramethylbenzidine	54827-17-7		c,d	5
Thiocid	5964-24-9	25–400	c,d	6
o-Toluidine	95-53-4		c,d	5
1,1,1-Trichloroethane	71-55-6		c,d	5
Trifluoroperazine [a]	117-89-5	200–4000 μM	b,c,d	8
Urethane	51-79-6		c,d	5

* All results are for chemicals tested in mitotic systems except where noted.

[a] Tested in meiotic system.

[b] Lack of appropriate controls; see criterion 4.

[c] Insufficient data to allow complete analysis; see criterion 5.

[d] Aneuploidy not confirmed; see criterion 6.

References: (1) Emeis, 1966; (2) Ferguson and Parry, 1984; (3) Kelly and Parry, 1983; (4) J.M. Parry, 1982; (5) J.M. Parry and Sharp, 1981; (6) J.M. Parry et al., 1979a; (7) J.M. Parry et al., 1981; (8) Sora and Bianchi, 1982; (9) Sora et al., 1982; (10) Sora et al., 1983.

stone of any valid investigation. Historical controls may be sufficient when it can be shown that the present data set is consistent with such a historical control. Data that show no effect (negative response) cannot be considered sufficient unless an acceptable positive control sample is included within the data set. Because these systems are genetically based, it is important to establish that the strains are responsive and have the original diagnostic genotype.

(3.1.4) Insufficient documentation

It may be acceptable in a review article to report only '+' or '−' for the sake of brevity, but such results cannot be evaluated. It is not possible to rely totally on data represented only graphically without support of raw data unless the positive effect is very large and obvious.

(3.1.5) Genetic validation

Chromosomes of *S. cerevisiae* can be observed directly only by techniques that are not suitable for screening purposes. Genetic events that mimic the aneuploid phenotype therefore must be considered. It is important to confirm that observation of a phenotype expected for aneuploidy is actually due to chromosome malsegregation and is not merely consistent with aneuploidy. Such confirmation might include detection of aneuploidy of additional chromosomes, aberrant tetrad ratios after meiosis, or observation of additional recessive markers on a monosomic chromosome.

(3.2) Test results

Several tests have been reported that meet all of the above criteria; these are presented in Table 1. In analyzing the literature, our criteria for acceptance or rejection of data have been strict and rigidly applied. Several chemicals tests met many but not all the criteria discussed above (also see Dellarco et al., this volume); these tests were judged to be inconclusive and are presented in Table 2. We feel that future efforts for monitoring chemicals for aneuploidy induction should use approaches which minimize the possibility of both false positive and false negative results.

Formal statistical analyses generally were not presented in the studies accepted. A positive response typically was characterized by multiple doses that produce aneuploid frequencies 3–4 times greater than the control. Further evidence was provided by reproducibility of the experimental results. We recommend that future studies provide more information about the variability in the systems in use and include more formal statistical analyses.

(4) Discussion and conclusions

Among the chemicals that were shown to induce aneuploidy are a number that apparently do not induce other genetic events (e.g., acetone, bavistan, nocodazole, TPA; Zimmermann et al., 1984; J.M. Parry et al., 1981; Wood, 1982). Such observations might be hypothesized to result from interaction of test chemical with target molecules other than DNA, those that uniquely serve to ensure proper segregation of chromosomes during cell division. Tubulin and microtubules certainly fall into this category. Nocodazole and bavistan are known to react specifically and covalently with tubulin. Organic solvents, such as acetone, might alter the solvation coat of the tubulin molecule and by this means, interfere with the proper polymerization into microtubules and maintenance of the steady-state polymers (Mitchison and Kirschner, 1984) or the proposed 'treadmilling' process (Margolis and Wilson, 1978). Further evidence that tubulin is a target comes from the observation that an intermittent cold storage period enhances aneuploidy induction by solvents (Zimmermann et al., 1984, 1985); it is well known that microtubules disassociate into their tubulin subunits in the cold and will reassemble when warmed. Other intracellular structures such as kinetochores, nuclear membranes, spindle plaques or centromeres might be specifically affected by chemicals, resulting in aneuploidy induction.

Aneuploidy is also induced by some well-known chemical mutagens such as methyl methanesulfonate and cyclophosphamide (Table 1), as well as by UV light and X-rays. Because these agents also induce other genetic effects such as mutation and recombination, there may be a relationship and/or common events between these various genetic changes. Some evidence for a relationship has been obtained in *S. cerevisiae*. High frequen-

cies of numerical chromosome abnormalities is one phenotype resulting from mutations in a number of functionally, diverse genes, including those involved in DNA repair (Section 1). A possible role for induced recombination leading to aneuploidy has also been reported. The mechanisms by which recombination defects or other mutant phenotypes could result in malsegregation of chromosomes are unknown and would seem to offer a fertile area for future investigation (Mason and Resnick, 1985).

An important DNA target could be that which has been identified as centromere DNA. It is possible to isolate from yeast DNA sequences that are essential to normal chromosome stability (Clarke and Carbon, 1980; Blackburn and Szostak, 1984). Genetically, they correspond to the centromere region. The sequences required for normal segregation are less than 300 base pairs in length and appear to be associated with unique protein structures (Bloom et al., 1984). Within the centromere regions, there are 3 consensus sequences between the different centromere DNAs that have been isolated. Given that the centromeric DNA is essential for proper chromosome segregation, DNA damage in this region might be expected to be particularly effective in inducing aneuploidy. Damage induced by UV in centromeric DNA has been measured recently (Resnick and Bloom, 1984), but the relation between damage and genetic consequence remains to be determined.

Reflection upon the present state of development of aneuploidy test systems in *S. cerevisiae* leads to suggestions for future research efforts. It would be useful to be able to observe events in more than one chromosome or pair of homologous chromosomes. Greater experience is needed in the development and use of chromosome gain detection systems and in systems to detect aneuploidy during meiosis. It is not known whether differences in chemically induced aneuploidy might exist between the various systems, which would suggest unique mechanisms of segregation. Finally, a much more extensive and reliable data base of tested chemicals should be generated, representing a broad diversity of structural types. Such information could be useful for identifying new aneuploidy-inducing agents and also provide insight into cross-species comparisons. Tubulin is a highly conserved molecule from one species to another. Yet, even with the present limited data base, slight differences seem to be sufficient to cause some chemicals to be negative in some systems and positive in others.

It is premature to recommend a particular treatment that could be presumed to be adequate and appropriate for all test chemicals. Several dose levels of a test chemical normally are used in anticipation of obtaining a dose–response relationship. The limited data available suggest to us that the effective dose range for aneuploidy induction can be very narrow. If the apparatus responsible for the proper segregation of chromosomes at cell division is the specific target, too little test chemical may give no effect while slightly more may result in inability of the cell to divide at all. There is general agreement that yeast cells under growing conditions are highly sensitive to chemical mutagens. A requirement for growth during treatment is probably more critical for aneuploidy than for other genetic end points, because chromosome segregation must occur in order to detect the event efficiently. On the other hand, there is good reason to believe that aneuploid cells would be selected against in a growing population of primarily euploid cells (J.M. Parry and Zimmerman, 1976; Mortimer et al., 1981). Therefore, the amount of growth permitted might be critical for determination of aneuploidy. Media composition will influence the growth rate of a culture and may also modify the efficacy of some chemical mutagens. Recent data (Zimmerman et al., 1984, 1985) show that aneuploidy induction by certain chemicals can be greatly enhanced when the treatment period in growth medium at 28°C is interrupted by a holding period at 4°C. The extent to which such a treatment is broadly applicable and its relevance beyond increasing the sensitivity of the test are presently unknown.

We feel that the proper assessment of chemically induced aneuploidy includes adequate genetic verification of the observed events. Because of our familiarity with strain D6.1M, we were able to identify some possible sources of artifacts and offer solutions to avoid such problems. Other yeast aneuploidy test systems also may yield misleading results; however, the present lack

of data does not allow these to be determined.

Overall, yeast systems are in many ways ideal for detecting aneuploidy and for relating chromosome malsegregation to other genetic end points. It is possible to follow events in haploids, diploids, and under conditions of meiotic development. As an organism for investigation, yeast is particularly useful, since mutants are available for probing the mechanisms of chromosome stability and for molecular investigations using current molecular biology techniques (Mason and Resnick, 1985).

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